Title

Cooperative breeding favours maternal investment in size over number of eggs in spiders

Running title

Egg investment strategies in cooperative spiders

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Abstract

The transition to cooperative breeding may alter maternal investment strategies depending on density of breeders, extent of reproductive skew and allo-maternal care. Change in optimal investment from solitary to cooperative breeding can be investigated by comparing social species with non-social congeners. We tested two hypotheses in a mainly semelparous system: that social, cooperative breeders, compared to subsocial, solitarily breeding congeners, 1) lay fewer and larger eggs because larger offspring compete better for limited resources and become reproducers; 2) induce egg size variation within clutches as a bet-hedging strategy to ensure that some offspring become reproducers. Within two spider genera, *Anelosimus* and *Stegodyphus,* we compared species from similar habitats and augmented the results with a mini-meta-analysis of egg numbers depicted in phylogenies. We found that social species indeed laid fewer, larger eggs than subsocials, while egg size variation was low overall, giving no support for bet-hedging. We propose that the transition to cooperative breeding selects for producing few, large offspring because reproductive skew and high density of breeders and young create competition for resources and reproduction. Convergent evolution has shaped maternal strategies similarly in phylogenetically distant species and directed cooperatively breeding spiders to invest in quality rather than quantity of offspring.

Introduction

The transition to cooperative breeding is likely to alter the selective pressures on maternal strategies as parental care transforms into allo-parental care (Russell and Lummaa 2009). Several factors may influence how cooperatively breeding females invest optimally in their offspring. Firstly, the density of breeders and the amount of available resources determines whether juveniles compete for limited resources. Theoretical models by Brockelman (1975) and Parker and Begon (1986) show that optimal maternal investment is to produce few, large offspring when siblings and non-siblings compete in a limited resource space, provided that larger offspring have a competitive advantage over smaller ones. Large clutches may further present a cost because more offspring may be more difficult for parents to tend and defend (Kam et al. 1998; Fox and Czesak 2000). These density-related effects could result in fewer offspring actually reaching the breeding age, which means that females producing fewer and larger offspring may gain higher fitness (Lack 1947; Noordwijk and Jong 1986; Godfray et al. 1991).

Secondly, cooperative breeding is usually characterised by some degree of reproductive skew (Keller and Reeve 1994). If larger offspring have a greater chance of becoming reproducers within a group, selection could also favour production of large offspring on the expense of offspring number (Brockelman 1975; Parker and Begon 1986). However, for many group living species, individuals' fitness is tightly linked to colony size, as larger colonies show lower risk of total group failure (e.g., Clutton-Brock et al. 1999; Kokko et al. 2001; Bilde et al. 2007). Hence, in species where offspring stay in their natal colony as additional group members, selection would disfavour producing very small clutches. In this case, where selection favours both large offspring and large clutch size, reproductive resources might be

allocated differentially into offspring to ensure that at least some will be large enough to become reproducers and that the group will be large enough to survive (diversified bet-hedging, e.g. Slatkin 1974; Philippi and Seger 1989; Einum and Fleming 2004). Maternal strategies in some animals do indeed include investing differentially in offspring within the same brood (Forbes 1999; Fox and Czesak 2000; Gibbs and Van Dyck 2009). This can be achieved, amongst other things, by manipulating egg size, nutritional packaging, or hatchling provisioning (e.g., Howe 1978; Crean and Marshall 2009).

Thirdly, the probability that a female can produce a second clutch later, and the presence of non-reproductive helpers may alter optimal investment. A breeding female may strategically save resources for a subsequent breeding attempt by investing less in quality or quantity of her offspring as a plastic response if she has helpers at her nest that compensate for this decrease in fecundity (Russell et al. 2007; Taborsky et al. 2007; Russell and Lummaa 2009). This strategy, however, is not available for semelparous females that breed only once in a lifetime as they would not gain by saving reproductive resources for future reproduction.

We have learned from previous studies that alterations to social environments can induce a plastic response on maternal investment within some cooperatively breeding birds and fish, and that cooperative breeding across bird species correlates negatively with clutch size (Arnold and Owens 1998; Russell et al. 2007; Taborsky et al. 2007). Here we take a different approach and investigate changes in maternal egg investment strategies that accompany the actual transition from solitary to cooperative breeding. Social spiders present an excellent system for testing maternal effect theories in the evolution of group living and cooperative breeding for several reasons: 1) Social spiders show allo-maternal care and reproductive skew: Less than half of all females in a colony reproduce, while the remaining females act as helpers (Vollrath 1986; Salomon and Lubin 2007; Salomon et al. 2008), and larger females

are considered to be the ones that become reproducers (Vollrath and Rohde-Arndt 1983; Rypstra 1993; Salomon et al. 2008; Grinsted and Bilde 2013). Allo-maternal care from mothers and helpers includes tending egg sacs and feeding hatchlings (Christenson 1984; Salomon and Lubin 2007). In the genus *Stegodyphus* allo-maternal care is suicidal: the young finally consume their mother and all adult females of the colony (Seibt and Wickler 1987). 2) Permanent sociality has evolved from subsocial congeners multiple times within distantly related spider families, and subsociality is still common allowing for comparative studies of social spiders and their ancestral state (Kullmann 1972; Kraus and Kraus 1988; Agnarsson 2006; Agnarsson et al. 2006; Johannesen et al. 2007). Subsocial spiders provide similar extended maternal care as social spiders, but juveniles show only a short period of cooperation in prey capture and feeding in the maternal nest before they disperse to live and breed solitarily (Avilés 1997; Lubin and Bilde 2007). Comparing traits from subsocial and social congeners can reveal valuable insights into the evolutionary consequences of the transition to permanent sociality and cooperative breeding from their subsocial ancestry. Comparing independent evolutionary origins of sociality from different spider genera may reveal evidence of convergent evolution and thus expose more general selective pressures on maternal investment. 3) Social and subsocial spiders usually reproduce only once in a lifetime (Lubin and Bilde 2007) so females need to optimise their investment in one clutch of eggs. Thus, the change in optimal maternal investment that accompanied the transition to cooperative breeding can be directly observed by comparing clutches of eggs from subsocial and social species.

We tested two not mutually exclusive hypotheses in the present study: First, if the transition to permanent group living and cooperative breeding was associated with competition among juveniles for resources and reproductive roles we would expect that social females produce clutches of fewer, larger eggs than their subsocial congeners. Second, if social spider females

use a bet-hedging egg-laying strategy to ensure that at least some of their own offspring become reproducers in the colony, we would expect that a social female produces a higher variation in egg sizes than a subsocial congener.

We investigated these hypotheses in two spider genera *Anelosimus* (Theridiidae) and *Stegodyphus* (Eresidae) to elucidate potential convergent evolution of maternal strategies. Both genera contain multiple independently derived social species that have evolved in distinctly contrasting habitats: Central and South American rainforest (*Anelosimus*) versus arid, open bush lands in Africa, the Middle East and India (*Stegodyphus*) (Kraus and Kraus 1988; Agnarsson et al. 2006; Johannesen et al. 2007; Lubin and Bilde 2007). Within each genus we compared maternal egg investment strategies of one social species with that of one or two closely related subsocial species that occurred in similar geographical regions and habitats. This allowed us to focus on evolutionary effects on maternal strategies while minimizing potentially confounding environmental effects. Based on previously published studies, we furthermore conducted a mini meta-analysis on egg numbers in an additional nine species and have presented these data in reconstructed phylogenies of the two genera to augment the data in the present study.

Methods

Study organisms

Social spiders live permanently in social groups where females cooperate in prey capture and feeding, web building and web maintenance, and brood care (Bilde and Lubin 2011). Due to a lack of pre-mating dispersal, social spiders breed with family members resulting in inbreeding and high levels of relatedness within colonies (Johannesen et al. 2002).

Anelosimus eximius occurs in the Americas from Panama to Argentina (Platnick 2012) where they form colonies of up to many thousands of individuals. Hence, *A. eximius* colonies are the largest of any social spider, although solitarily breeding females can occasionally be found (Vollrath 1982; Avilés 1997). Breeding occurs year round and thus colonies contain spiders of all instars through most of the year (Aviles 1986; Avilés 1997). Social *A. eximius* and subsocial *A. baeza* belong to the *eximius* group in the *Anelosimus* phylogeny (Agnarsson 2006) and thus are valid as a sister clade comparison. Additionally, spiders from both species used in this study were found in the same area in similar habitats. *Anelosimus baeza* occurs from Panama to Peru (Platnick 2012) where it mostly breeds solitarily, but can be found to live in multi-female colonies (L. Grinsted, *personal observation*). However, the level of cooperation, if any, within these colonies is unknown (Agnarsson 2006). Females of some subsocial *Anelosimus* species can produce two egg sacs in their life time (I. Agnarsson, *personal communication*), whereas others seem to only produce a second egg sac if the first one is abandoned (Marques et al. 1998). Females of social *Anelosimus* species are thought to usually produce only one egg sac in their life time although some might produce two (Aviles and Salazar 1999). *Anelosimus* spiders live for up to a year (Aviles and Tufino 1998).

Social *S. sarasinorum* occurs in India, Sri Lanka and Nepal (Platnick 2012) in dry, shrubby habitats. Nests contain one female to several hundreds of individuals. The subsocial *S. tibialis* and *S. pacificus* also occur in India, and the individuals used in this study were found in the same area and in similar habitat as *S. sarasinorum*. *Stegodyphus pacificus* is the sister species of *S. sarasinorum* (Settepani et al., *unpublished data*) and, hence, is appropriate for a sister clade comparison of the effects of social level on maternal strategies. Social and subsocial *Stegodyphus* females produce only one egg sac in their life time, unless the egg sac is lost after which they may produce another one, and they die when the young eventually consume them (Jacson and Joseph 1973). As these spiders only live for about a year and occur in

seasonal habitats, breeding is restricted to one season in their lifetime and, hence, all spiders within colonies of social *Stegodyphus* spiders are approximately of the same age and life stage (Crouch and Lubin 2000; Lubin et al. 2009).

Collections and measurements

Stegodyphus

Nests refer to silken retreats consisting of either a single female with her single egg sac, or colonies with multiple females and multiple egg sacs. Nests of the subsocial *S. tibialis* (N_{nests} = 12, $N_{egg\ sacs}$ = 12) and *S. pacificus* (N_{nests} = 10, $N_{egg\ sacs}$ = 10) were collected in October to December 2010 near Kuppam in India (N 12°48.854' E 78°15.964'). In the same area, colonies of the social *S. sarasinorum* were collected in January and February 2012. Whenever possible, up to five egg sacs per nest of *S. sarasinorum* were sampled (N_{nests} = 11, $N_{egg\ sacs}$ = 30, median $N_{egg\ sacs/nest}$ = 2). Upon collection colonies were dissected and spiders were counted. Only females and males in the parental cohort were included in the colony size count; if colonies contained hatchlings, these were not included in the count. Colony sizes ranged from a single female to 106 spiders. An overview of species and sample sizes is available in the Online Supplementary Materials (Table OSM1).

The prosoma width of mothers was measured with a digital calliper (Toolmate) to the nearest 0.01 mm. Prosoma width is a widely accepted measure of body size in spiders as it is a sclerotized body part affected little by satiation state (Hagstrum 1971; Jakob et al. 1996). An estimate of mother size in multi-female colonies was obtained by taking the average prosoma width of up to 15 randomly chosen adult females in each colony (Bilde et al. 2007) or of all females in colonies smaller than 16.

Anelosimus

Egg sacs of the social *A. eximius* and the subsocial *A. baeza* were collected near Sumaco in Ecuador (S 00°43.492' W 77°38.665') in May and June 2011. The subsocial *A. baeza* sometimes formed multi-female nests, resembling social colonies, although adult females most likely did not cooperate in prey capture and brood care. Whenever possible, up to five egg sacs were sampled from nests of both *A. eximius* and *A. baeza* (*A. eximius*: $N_{nests} = 33$, $N_{egg\ sacs} = 136$, median $N_{egg\ sacs/nest} = 5$; *A. baeza*: $N_{nests} = 21$, $N_{egg\ sacs} = 30$, median $N_{egg\ sacs/nest} = 1$). An overview of sample sizes is available in the Online Supplementary Materials (Table OSM1).

Two measures of female body size were obtained: prosoma width and the combined length of tibia and patella of the first leg by measuring with a digital calliper to the nearest 0.01 mm either directly in the field or after collection of nests. The length of tibia + patella is recommended as a proxy for body size in *Anelosimus* spiders (e.g., Aviles 1986). The prosoma on *Anelosimus* spiders is relatively small (width: 0.9-1.7mm) and therefore susceptible for higher measurement error when measured by hand, whereas the length of tibia + patella on the first leg is more easily measured (length: 2.0-3.8mm). Estimates of mother size in social *A. eximius* was obtained by taking the average measure from up to 20 randomly chosen adult females in each colony or of all females in colonies smaller than 21. When egg sacs were collected from multi-female nests of *A. baeza*, it was clear which female the egg sac belonged to, as only one female was in close proximity of the egg sac, and so the actual mother was measured.

The size of an *A. eximius* colony was obtained either by dissecting the colony and counting the spiders (22 nests) or estimated based on the physical dimension of the nest (11 nests: see OSM: Additional Methods). Colony sizes across the 33 nests ranged from a single female to

1934 non-juvenile spiders (i.e., the number of subadult and adult males, and adult females and two different instars of subadult females). To determine the colony size of multi-female *A. baeza* nests, the transparent nests were visually inspected and adult and subadult females and males were counted. Colony sizes ranged from a single female to 54 non-juvenile spiders.

Egg number and egg sizes

In the lab, each egg sac was opened carefully and the eggs were spread out on a flat, black background. Broken and lost eggs were counted, and individual eggs with small, parasitic larvae attached were removed and counted. A photo was taken with a digital camera (Canon Cyber-shot DSC-W330 14.1 mega pixel) at a set distance to the background. Immediately afterwards a photo was taken of a piece of millimeter-scale paper for reference. The size of each egg in each of the clutches was measured, to nearest 0.01 mm^2, with the use of a custom-fitted macro for ImageJ 1.45 (Abramoff et al. 2004; Gibbs et al. 2010), contrasting the light-coloured egg against a black background. Egg numbers were obtained manually by counting eggs on each photograph and adding the number of lost or removed eggs for each clutch.

Egg number among species in phylogenies

To supplement our results, we performed a literature search on reproductive strategies in *Anelosimus* and *Stegodyphus* species. We were also able to collect egg sacs from yet an extra *Stegodyphus* species (*S. mimosarum*, 8 egg sacs collected from 2 nests in Madagascar, May 2012). In this way, we obtained egg numbers from an additional six *Anelosimus* and three *Stegodyphus* species. We then mapped the average egg number per egg sac from each species onto the phylogenies of the two genera so that closely related social and subsocial species

could be compared. This meta-analysis was mainly meant for descriptive analysis and not for phylogenetic contrast analysis as sample sizes were low and the phylogenies were lacking branch lengths.

Specifically, we obtained egg numbers from the following social species: *A. domingo* (Aviles and Maddison 1991), *A. dubiosus* (Marques et al. 1998), *A. rupununi* (Aviles and Salazar 1999), *S. dumicola* (Aviles et al. 1999) and *S. mimosarum* (this study); and the following subsocial species: *A. jucundus* (Aviles and Maddison 1991), *A. jabaquara* (Gonzaga and Vasconcellos-Neto 2001), *A. studiosus* (Pruitt and Ferrari 2011), *S. lineatus* (Salomon et al. 2005). An overview of references and sample sizes is available in the Online Supplementary Materials (Table OSM2).

The as yet unpublished molecular phylogeny of *Stegodyphus* was based on nine independent nuclear loci (Settepani et al., *unpublished data*). The nuclear loci were amplified with 13 primers designed from alignments of *S. lineatus*, *S. tentoriicola* and *S. mimosarum* published in (Mattila et al. 2012). The best substitution model for each locus was estimated with PartitionFinder (Lanfear et al. 2012). The phylogeny was constructed using the Bayesian method implemented in MrBayes 3.2 (Ronquist et al. 2012b). MrBayes was run for five million generations with a sampling frequency of 500, a burn-in of 25% and two chains. The partial *Anelosimus* phylogeny was based on the one published in Agnarsson (2006). The partial phylogenies presented here were drawn in TreeViewX.

Statistics

We used linear mixed effect models with the following three predictor variables: social level, mother size and colony size. Response variables used were egg size (all individual egg sizes), variation in egg sizes within egg sacs (one value per egg sac) and egg number (one value per

egg sac). The *lmer* function from the lme4 package (Bates et al. 2011) in R (version 2.14.2) was used (R Development Core Team 2011). For models with a Gaussian error structure we checked whether the assumptions of normally distributed and homogenous residuals were fulfilled by inspecting *qq*-plots and the residuals plotted against fitted values. In each of these models the response variable was transformed to optimize normality and homogeneity of the residuals (the different transformations are apparent in the Results, and presented in the Online Supplementary Materials: Description of Statistical Models). We based model fitting on Maximum Likelihood rather than the default option of Restricted Maximum Likelihood tests in these models. We used a Poisson error distribution with a log link function in models where egg number was the response variable and established that data was not overdispersed before proceeding.

For all constructed models we confirmed that the model was robust and that there were no data points with a disproportionally large effect. We did this by excluding data points one by one and comparing the range of estimated coefficients derived with those obtained from the full model. We also determined the variance inflation factor for full models (reduced to lms by excluding random effects) using the vif function from the *car* package (Fox and Weisberg 2011), ensuring the models did not suffer from multicollinearity. *p*-values were obtained by using likelihood ratio tests (χ^2) to compare full models with reduced models in which the main effect in question had been omitted. When continuous predictor variables were included in an interaction term, they were *z*-transformed to a mean of zero and a standard deviation of one in order to facilitate interpretation of parameter estimates. The overall significance of the full model was established by comparing it to a null model that included all random effects and random slopes. Only if the full model was significant we proceeded to test the significance of interaction terms and main effects. Only if interaction terms were found non-significant, the significance of the main effects involved in the interactions were tested.

For each of the three response variables we started out by testing the effect of social level (i.e., social versus subsocial) across genera by including all five species in the models. As female body size varies greatly among and within species, mother size was accounted for in the models by including it as a covariate. The interaction between social level and mother size was included to allow for the possibility that mother size had different effects on the response variable according to social level.

If any of the predictors showed a significant effect in tests including all five species, post hoc tests were performed with similar models testing the same predictors within genera. Colony size could be included into the models testing *Anelosimus* spiders, as both the social and the subsocial *Anelosimus* species formed colonies. The effect of colony size was tested separately for the social *S. sarasinorum* (rather than in a test including all three *Stegodyphus* species) as the two subsocial *Stegodyphus* species always occurred solitarily. The effect of colony size was further examined in post hoc tests performed on each *Anelosimus* species by itself.

Whenever relevant, random effects and random slopes were included in the models. In some models, random slopes of mother size amongst genera, and mother size amongst species, were used. These allowed for random variation in the slope of the correlation between mother size and response amongst species and amongst genera.

We also examined the trade-offs between egg size and egg number at the level of individual mothers in each species separately. We did this by building GLMs with a negative binomial error distribution to account for overdispersion with egg number as the response variable and average egg size within egg sacs and mother size as predictor variables.

When including mother size in models containing all five species or only *Stegodyphus* species, prosoma width of females within colonies were used as a proxy for mother size. In

models testing only *Anelosimus* species, length of tibia + patella was used as a proxy for mother size.

Detailed descriptions of all models tested are available in the Online Supplementary Materials (Description of Statistical Models).

Based on the meta-analysis we compared mean egg number from social species with that from their appropriate subsocial congener in a Wilcoxon test for matched pairs (see Table OSM3 for details on the species pairs).

All raw data is available at Dryad Digital Depository (doi: 10.5061/dryad.t6k57).

Results

Does social level predict egg size and egg number?

Social spider species laid larger eggs than subsocial congeners overall (Table 1 and Fig. 1a): level of sociality significantly predicted egg size in a model including all five species, but this was dependent on mother size (i.e., significant interaction between social level and mother size; Table 1). Specifically, post hoc tests within genera showed that while social level had a significant effect on egg size within both genera, mother size also significantly predicted egg size only within *Stegodyphus*, not within *Anelosimus*. Larger *Stegodyphus* females laid smaller eggs in both social and subsocial spiders (Table 1 and Fig. 1a). Colony size significantly positively correlated with egg size within both *Anelosimus* species (Fig. 2c and 2e), while this correlation was not significant in *S. sarasinorum* (Fig. 2a).

Social spider species laid significantly fewer eggs than their subsocial congeners in a model containing all five species (Table 2a and Fig. 1b). Mother size had a significant, positive

effect on egg number overall (Table 2a). Within *Stegodyphus* species, this effect of mother size was highly significant (Fig. 1b and Fig. OSM1a) while social level only showed a close-to significant effect (Table 2a). In *Anelosimus* both female size and social level significantly predicted egg number, dependent on the size of the colony (significant interaction between social level and colony size; Table 2a). This means that apart from larger mothers laying more eggs in both *Anelosimus* species (Fig. OSM1b), subsocial spiders laid more eggs than social spiders only in smaller colony sizes. When colony sizes reached its maximum for subsocial *A. baeza*, the egg numbers were similar to those of social *A. eximius* (Fig. 2d and 2f). Within each of the three group forming species, *A. eximius* was the only for which colony size had a significantly, positive effect on egg number (Table 2a and Fig. 2b, 2d and 2f).

Social species laid significantly fewer eggs (grand mean 37.9 eggs per egg sac) compared to subsocial species (grand mean 127.8 eggs per egg sac) in sister clades (Table OSM3) in both *Stegodyphus* (Fig. 3) and *Anelosimus* (Fig. 4) (Wilcoxon for matched pairs, $p = 0.0078$).

We found a highly significant negative correlation between egg number and egg size only in the subsocial *A. baeza* (Table 2b and Fig. OSM2) when mother size was taken into account. We also saw a negative correlation in subsocial *A. pacificus*, but this trend was not significant, and in the remaining three species we found no correlation (Table 2b).

Does social level predict variation in egg sizes within clutches?

The variation of egg sizes within egg sacs of social species was not different to that of their subsocial congeners within both genera. Social level and *z*-transformed mother size had no effect on the coefficient of variation in egg sizes within egg sacs (log transformed CV) among the five species (*ChiSq* = 6.30, *d.f.* = (8, 5), $p = 0.10$, $N_{egg\ sacs} = 211$, $N_{nests} = 85$, Fig. 1c). Square root transformed colony size also did not affect the variation in egg sizes within

egg sacs (log CV) in the three colony forming species, *S. sarasinorum* (*ChiSq* = 0.87, *d.f.* = (4, 3), $p = 0.35$, $N_{egg\ sacs} = 29$, $N_{nests} = 11$), *A. eximius* (*ChiSq* = 0.05, *d.f.* = (4, 3), $p = 0.82$, $N_{egg\ sacs} = 132$, $N_{nests} = 32$) and *A. baeza* (*ChiSq* = 0.02, *d.f.* = (4, 3), $p = 0.88$, $N_{egg\ sacs} = 30$, $N_{nests} = 21$). Additionally, colony size in *A. eximius* did not affect the within-colony variation in average egg sizes per egg sac (*rho* = -0.24, $p = 0.25$) or the within-colony variation in egg number (*rho* = -0.18, $p = 0.38$).

Discussion

We found support for the hypothesis that with the transition from solitary to cooperative breeding maternal investment strategies have been altered to invest in size of offspring on the expense of number. Social, cooperatively breeding spiders laid significantly fewer, larger eggs than their subsocial congeners, when factoring out the effect of mother size. Our results suggest that the selection for laying fewer, larger eggs have acted similarly on females within two separate genera of spiders that evolved sociality in distinctly different geographical and environmental circumstances. Hence, we propose that convergent evolution has shaped maternal egg investment strategies in social spiders, likely due to competition among offspring for resources and reproduction in the transition to cooperative breeding. This may indicate that the transition to cooperative breeding presents similar selective pressures more generally and that these may be applicable for a wide range of cooperatively breeding organisms. We found no support for the hypothesis that social spider females invest differentially in eggs within clutches. The variation of egg sizes within egg sacs was very low in both social and subsocial species, suggesting that females do not induce size variation amongst their offspring at the egg stage as a bet-hedging strategy.

Maternal strategies in the evolution of cooperative breeding

Theoretical models predict that there is an optimal balance between offspring number and size in animals (Smith and Fretwell 1974) and that the optimal egg size increases, while egg number decreases when competition among siblings and non-siblings increases (Brockelman 1975; Sargent et al. 1987). Our results support these theoretical predictions as social spiders laid fewer, larger eggs than their subsocial congeners. In social spider colonies there may be high densities of breeding females and both siblings and non-siblings might have competed for limited resources in the transition to cooperative breeding. Larger offspring may have had a competitive advantage and, hence, grown enough to reproduce while smaller group members would fail to do so (Vollrath 1986; Lubin 1995; Ulbrich and Henschel 1999; Whitehouse and Lubin 1999). Hence, selection for producing large offspring may have been strong, even on the expense of offspring number. A comparable example may be found in the communally breeding banded mongoose, *Mungos mungo*, where young in large colonies compete for allo-maternal care. Larger, heavier young have higher competitive abilities and obtain more care and thus benefit from a significantly higher survival rate making them more likely to grow up to reproduce (Hodge et al. 2009). Hence, giving birth to larger pups is advantageous.

Cooperative breeding in animals is associated with costs and benefits that may influence how mothers optimally invest in their offspring. In social spider colonies, individual risk of mortality is diminished due to both a lack of dispersal and benefits of group living. These benefits include increased protection against predators when living permanently in a large, protective nest (Bilde et al. 2007; Lubin and Bilde 2007), and extensive brood care from mothers and helpers. Allo-maternal care provide clear fitness benefits to young in the form of higher survival and growth rates (Salomon and Lubin 2007). In mammals, fitness benefits of

receiving help when reproducing also include increased growth and survival of offspring and allows for decreased inter-litter intervals as females can breed more than once in their lifetime (Jennions and Macdonald 1994; Russell et al. 2003). Across bird species, cooperative breeding is associated with clutches of fewer eggs (Arnold and Owens 1998; but see Cockburn 2003), and in some fish, females lay clutches of smaller eggs when more helpers are present (Taborsky et al. 2007). This apparent lower fecundity in cooperatively breeding birds and fish seem to be compensated by significantly lower mortality rates due to saving of resources, allowing for the production of more clutches later in life (Arnold and Owens 1998; Taborsky et al. 2007). Small egg numbers in social spiders is often interpreted as a cost of group living on the reproductive output of individual spiders (Aviles and Tufino 1998; Bilde et al. 2007). Indeed, if social spiders as compared to subsocial spiders laid fewer eggs of a similar size, this could have been interpreted as a cost of cooperative breeding to reproductive output. However, our novel results suggest that investing in fewer offspring is compensated by an increase in egg size, and hence, may be an adaptation to, rather than a cost of, cooperative breeding and group living. That the altered maternal strategy is in fact an evolutionary response to social living is supported by our egg size data. Indeed, there was no overlap between the observed egg sizes of social females and that of their subsocial congeners even though social and subsocial female body sizes overlapped within both genera (Fig. 1a).

We found clear evidence of a trade-off between egg size and egg number in one species only, the subsocial *A. baeza*, out of the five species examined. Trade-offs between egg size and number may be difficult to detect in species that use adult-acquired resources for reproduction or show parental care, because total reproductive effort is then difficult to quantify (Bernardo 1996; Fox and Czesak 2000). This is the case for subsocial spiders and to

an even greater extent for social species with allo-maternal care, which may explain why we could not detect the trade-off in the remaining species.

Maternal influence on size variations in offspring

We found no difference in egg size variation within egg sacs between social spiders and their subsocial congeners, suggesting that social spider females do not induce size variation in their offspring at the egg stage. Recent evidence indicates that body size variation amongst group members in social spiders may be induced at an early life stage and remain more or less stable throughout the colony life cycle (Grinsted and Bilde 2013). These body size hierarchies lead to partitioning of reproduction, and if they arise at an early life stage, maternal effects might play a role in assigning reproductive roles in offspring. Although we found no evidence to suggest that females vary the sizes of their offspring at the egg stage, differential maternal investment in offspring can occur at later stages (Russell and Lummaa 2009). Maternal and allo-maternal feeding of young allows for potential maternally induced size differences among hatchlings by differential feeding. Alternatively, unsynchronized hatching of egg sacs could create these differences, as earlier hatched offspring would get a head start in weight gain (Laaksonen 2004). Further research on post-hatching maternal effects and hatching asynchrony will add to the understanding of the mechanisms behind partitioning of reproductive roles in social spider colonies.

Phylogenetic differences

Our results suggest similarities in maternal egg investment strategies in the convergent evolution of sociality in two phylogenetically distant spider genera. These two genera contain spiders with distinctly different natural histories and origins of sociality. Social and many subsocial *Anelosimus* species occur in non-seasonal rain-forest habitats in the New World

(Agnarsson et al. 2006), whereas social and subsocial *Stegodyphus* species occur in seasonal, arid, open shrub lands in the Old World (Kraus and Kraus 1988; Majer et al. 2013). Female *Anelosimus* spiders can usually reproduce throughout the year, and might produce a second egg sac after the first one (although it is unclear how often this occurs, Marques et al. 1998; Aviles et al. 2007). This means that resources obtained for egg production might not all be allocated to one brood, but may strategically be divided relative to: future reproductive opportunities depending on environmental conditions, the predicted survivability of the first brood, and the probability of reproducing a second time. This situation is different for *Stegodyphus* species that only lay a second egg sac in case the first one is lost (Jacson and Joseph 1973). These differences in life history traits between the two genera may explain why we found stronger associations between mother size and egg number in *Stegodyphus* spiders compared to *Anelosimus* spiders, and also why mother size did not correlate with egg size in *Anelosimus*, while it did in *Stegodyphus*.

Another difference observed between the social species *A. eximius* and *S. sarasinorum* was the effect of colony size on reproductive output. In *A. eximius* both egg size and number increased in larger colonies, while this effect was lacking in *S. sarasinorum*. Hence, living in larger groups seems to pose benefits to social *Anelosimus* but not to social *Stegodyphus*. It is possible that the extraordinarily high colony sizes reached in *A. eximius* (nest members in the thousands) mean higher assurance of offspring survival due to more allo-mothers and higher colony survival. Females may subsequently allocate more of their body resources into egg laying and less into maternal care. While subsocial *A. baeza* also showed an increase in egg size in larger colonies, this was accompanied with a (not significant) decrease in egg number. Subsocial spiders normally live solitarily, and *A. baeza* may not have adapted to group living in the same way as social *Anelosimus* species have. Thus, they may not enjoy the same benefits such as increased egg number when forming groups. However, as these spiders do

occasionally occur in groups, perhaps they experience other benefits to group living such as higher protection within colonies leading to higher offspring and colony survival. Other aspects of group living appear to be similar between the social *Anelosimus* and *Stegodyphus*, including higher offspring survival and increased colony-level survival (Aviles and Tufino 1998; Bilde et al. 2007).

Conclusion

Cooperative breeding is likely to alter optimal maternal strategies. In mammals, birds and fish, the presence of allo-mothers may allow breeders to produce more clutches, which in turn can affect how females invest in size and number of offspring within clutches (Jennions and Macdonald 1994; Arnold and Owens 1998; Russell et al. 2003; Taborsky et al. 2007). Cooperatively breeding spiders commonly produce only one clutch in their lifetime, and hence need to optimise investment in this one clutch. In accordance with theoretical predictions, we show that cooperatively breeding social spiders within two different genera produce clutches of fewer, larger eggs than those of their subsocial, solitarily breeding congeners representing their ancestral state. We propose that this altered maternal investment strategy may reflect selection for producing larger offspring that are more likely to become reproducers due to a competitive advantage over smaller group members. Finally, we show that clutches of eggs in social and subsocial species showed similar low variation in egg sizes, suggesting that mothers do not induce size variations in their offspring at the egg stage as a bet-hedging strategy. Our study shows how convergent social evolution can shape maternal strategies similarly in phylogenetically distant species, and highlights how permanent group living and cooperative breeding can direct maternal investment from quantity to quality of offspring.

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DATA ARCHIVING

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Table 1 Results from GLMMs testing the effect of various predictors on the response variable egg size. Test values and *p*-values are given in the right three columns. Significant *p*-values are highlighted in bold. The word "transformed" is shortened to "trans"

Table 1

Egg size (individual egg sizes, log trans)

GLMMs of the effect of social level and colony size		*ChiSq*	*d.f.*	*p-value*
Across genera (5 species, 85 nests 13282 eggs, 213 egg sacs)	social level * *z*-trans prosoma width	*5.07*	*11, 10*	***0.024***
Within *Stegodyphus* (3 species, 32 nests, 6407 eggs, 50 egg sacs)	social level * *z*-trans prosoma width	*0.90*	*9, 8*	*0.34*
	social level	*6.35*	*8, 7*	***0.012***
	z-trans prosoma width	*5.19*	*8, 7*	***0.023***
Within social *S. sarasinorum* (29 egg sacs)	full model (colony size + prosoma width)	*5.34*	*5, 3*	*0.069*
Within *Anelosimus* (2 species, 52 nests, 6632 eggs, 157 egg sacs)	social level * *z*-trans length of tibia+patella	*1.69*	*9, 8*	*0.19*
	social level * colony size	*1.47*	*8, 7*	*0.22*
	social level	*123.1*	*6, 5*	***<0.0001***
	z-trans length of tibia+patella	*0.81*	*7, 6*	*0.37*
	colony size	*3.04*	*6, 5*	*0.081*
Within social *A. eximius* (127 egg sacs)	colony size	*9.08*	*5, 4*	***0.0026***
	length of tibia+patella	*2.33*	*5, 4*	*0.13*
Within subsocial *A. baeza* (30 egg sacs)	colony size	*5.59*	*5, 4*	***0.018***
	length of tibia+patella	*0.66*	*5, 4*	*0.42*

Table 2 Results from models testing the effect of various predictors in the response variable egg number. Test values and *p*-values are given in the right three columns. Significant *p*-values are highlighted in bold. The word "transformed" is shortened to "trans". a) shows results from GLMMs. b) shows results from GLMs on each separate species. In these models both mother size and average egg size were included as main effects although effects of mother size is not depicted here as they are similar to those depicted in the results from the GLMMs

Table 2

Egg number (eggs per egg sac)

a) GLMMs of the effect of social level and colony size (controlled for colony ID)		*ChiSq*	*d.f.*	*p-value*
Across genera (5 species, 212 egg sacs, 86 nests)	social level * *z*-trans prosoma width	*0.28*	*9, 8*	*0.59*
	social level	*4.21*	*8, 7*	***0.04***
	z-trans prosoma width	*6.87*	*8, 7*	***0.0088***
Within *Stegodyphus* (3 species, 51 egg sacs, 33 nests)	social level * *z*-trans prosoma width	*0.87*	*7, 6*	*0.35*
	social level	*3.61*	*6, 5*	*0.057*
	z-trans prosoma width	*12.3*	*6, 5*	***0.00045***
Within social *S. sarasinorum* (29 egg sacs, 11 nests)	colony size	*3.21*	*4, 3*	*0.073*
	prosoma width	*22.3*	*4, 3*	***<0.0001***
Within *Anelosimus* (2 species, 157 egg sacs, 52 nests)	social level * *z*-trans length of tibia+patella	*0.38*	*7, 6*	*0.54*
	social level * colony size	*5.97*	*6, 5*	***0.015***
	z-trans length of tibia+patella	*7.64*	*6, 5*	***0.0057***
Within social *A. eximius* (127 egg sacs, 31 nests)	colony size	*7.25*	*4, 3*	***0.0071***
	length of tibia+patella	*8.02*	*4, 3*	***0.0046***
Within subsocial *A. baeza* (30 egg sacs, 21 nests)	full model (colony size + length of tibia+patella)	*4.28*	*4, 2*	*0.12*
b) GLMs of the association with egg size (mother size included in all models)		*z-value*	*d.f.*	*p-value*
Social *S. sarasinorum* (28 egg sacs)	average egg size	*-0.30*	*27, 25*	*0.76*
Subsocial *S. tibialis* (12 egg sacs)	average egg size	*0.85*	*11, 9*	*0.39*
Subsocial *S. pacificus* (9 egg sacs)	average egg size	*-1.81*	*8, 6*	*0.070*
Social *A. eximius* (131 egg sacs)	average egg size	*1.16*	*130, 128*	*0.25*
Subsocial *A. baeza* (30 egg sacs)	average egg size	*-2.79*	*29, 27*	***0.0053***

Fig. 1 Egg size (a), egg number (b) and variation in egg size (c) plotted against mother size (prosoma width, mm) of all five species. Egg size is the grand mean egg size (average egg sizes within egg sacs averaged within nests, mm^2). Variation in egg size is the coefficient of variation in egg sizes within egg sacs, averaged within nests. Egg numbers are eggs per egg sac averaged within nests. *Stegodyphus* species are represented as squares and *Anelosimus* as circles. The social species *S. sarasinorum* and *A. eximius* are represented by black points and full regression lines. Subsocial species are represented by grey and white points and dotted lines: *S. tibialis* and *A. baeza* are grey; *S. pacificus* is white. Regression lines have only been drawn for statistically significant associations between variables, although here they represent correlations performed on averaged data and do not directly reflect the results from the statistical models

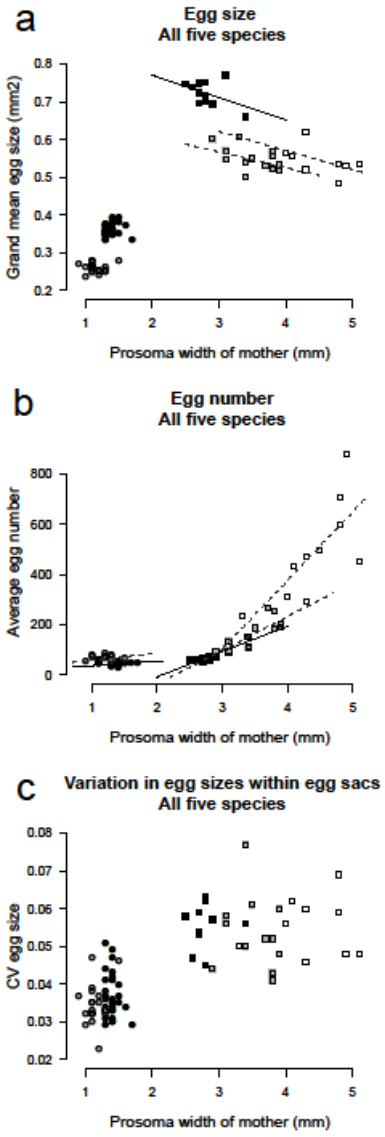

Fig. 2 Egg size (a, c, and e) and egg number (b, d, and f) plotted against colony size (number of non-juvenile spiders) for the three group forming species: social *S. sarasinorum* (a and b), social *A. eximius* (c and d), and subsocial *A. baeza* (e and f). Egg size is the grand mean egg size (average egg sizes within egg sacs averaged within nests, mm^2) and egg numbers are eggs per egg sac averaged within nests. Regression lines have only been drawn for statistically significant associations between variables, although here they represent correlations performed on averaged data and do not directly reflect the results from the statistical models

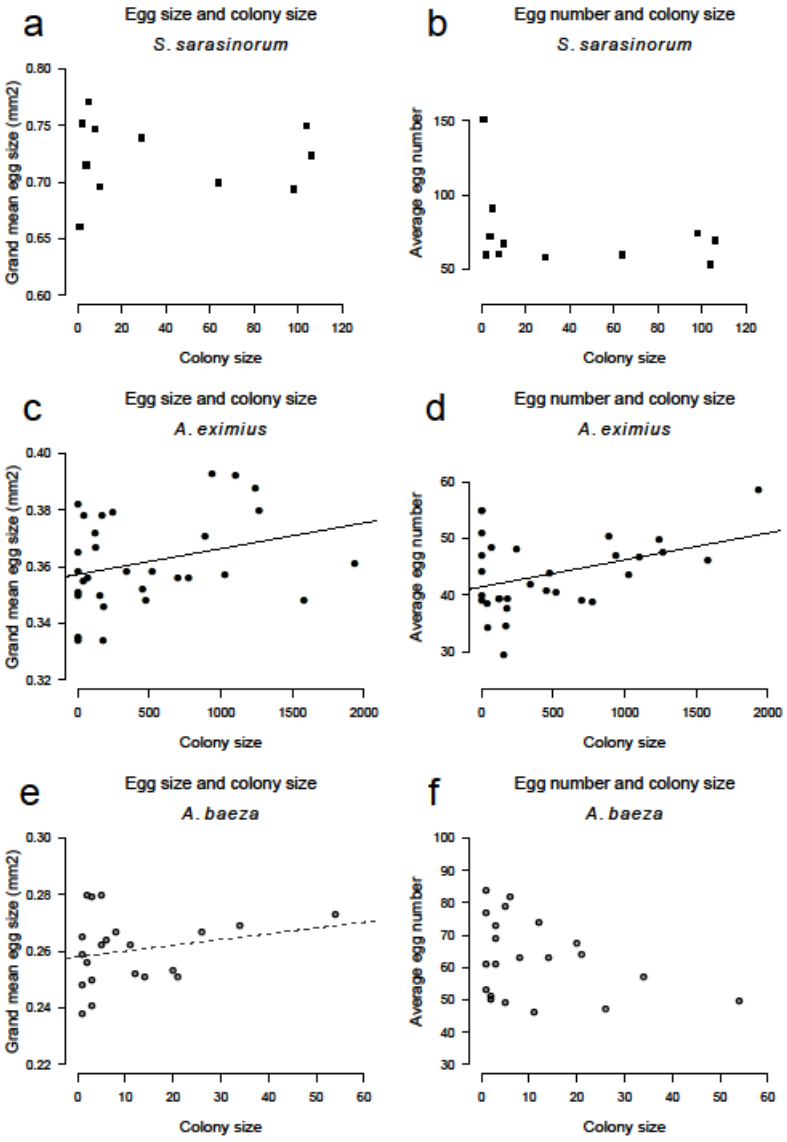

Fig. 3 Partial phylogeny of *Stegodyphus* with average egg number +/- standard deviation (rounded off to whole numbers; na indicates sd not available) given for the species from which data was available. Social species are presented in bold; the remaining species are subsocial. Stars indicate the species investigated in the present study. The tree represents topology only i.e., branch lengths do not indicate evolutionary distance. The topology is based on a preliminary molecular phylogeny from Settepani et al. (*unpublished data*) constructed using 13 independent nuclear loci and analysed with the Bayesian method implemented in MrBayes 3.2 (Ronquist et al. 2012)

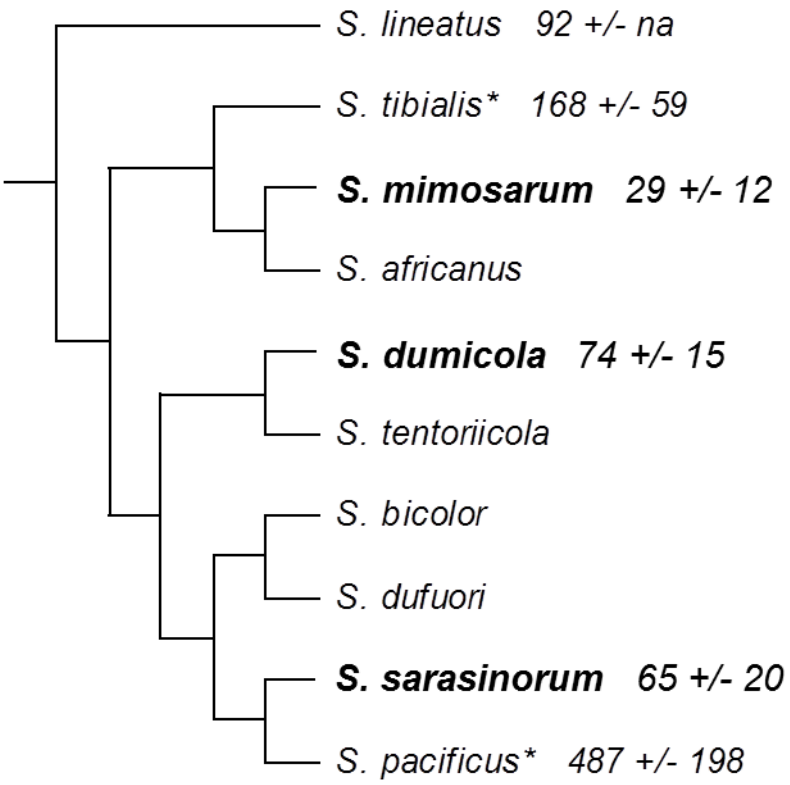

Fig. 4 Partial phylogeny of *Anelosimus* with average egg number +/- standard deviation (rounded off to whole numbers; na indicates sd not available) given for the species from which data was available. Social species are presented in bold; the remaining species are subsocial. Stars indicate the species investigated in the present study. When egg numbers were known from both solitary (sol.) and colony living (col.) females, two averages are given. The tree represents topology only i.e., branch lengths do not indicate evolutionary distance. The topology is based on the phylogeny published in Agnarsson (2006), which is a parsimony analysis of a morphological matrix (43 taxa, 147 characters)